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TITLE: HARNESSING GPR17 BIOLOGY FOR TREATING DEMYELINATING DISEASE

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14. ABSTRACT
Multiple sclerosis (MS) is a devastating demyelinating disease in the CNS. We have recently identified a new G-protein coupled receptor 17 (GPR17), whose activation was shown to inhibit myelination. In this study, we hypothesize that GPR17 signaling activation results in blockade of remyelination. The specific aims of this study are: (1) to delineate the role of GPR17 in murine MS models of demyelinating diseases; and (2) to test the therapeutic potential for GPR17 agonists and antagonists in MS models including cuprizone- and EAE- induced demyelination. During the funding period of this project, we used cuprizone-induced demyelinating animal model to analyze the GPR17 function in remyelination. We evaluated the dynamics of GPR17 expression, and examined control and GPR17 null mice over the course of demyelination and remyelination process. Our preliminary study showed that GPR17 deletion may have a protective role during cuprizone-induced demyelination and enhances remyelination as well as in EAE model of MS. Moreover, we found that GPR17 agonists inhibit OPC differentiation while GPR17 antagonists enhance oligodendrocyte differentiation in culture. These preliminary studies provide us a strong basis to pursue drug-based treatment of the demyelinating diseases.

15. SUBJECT TERMS- remyelination, G-protein coupled receptor, GPR17, demyelinating animal model, GPR17 agonist and antagonist

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Introduction

Multiple sclerosis (MS) is thought to be an immune-mediated disease that is characterized by an immune attack on myelin sheaths in the central nervous system (CNS). There are several immune-focused treatment strategies for this disease, all showing partial benefit. It is becoming increasingly clear that we need treatment options that also enhance the process of CNS repair ("remyelination") and thus, it is critical to understand the underlying mechanisms that promote or deter such remyelination. The bHLH transcription factor Olig1 promotes oligodendrocyte maturation and is required for myelin repair [1, 2]. Recently, we have discovered a G-protein coupled receptor 17 (GPR17), whose function is to oppose the action of Olig1 and acts as a negative regulator for OPC differentiation [3]. We observed that sustained expression of GPR17 resulted in myelination defects in transgenic mice, whereas GPR17 knockout mice developed early onset of myelination. In addition, blocking of GPR17 was reported to enhance brain recovery after traumatic brain and spinal cord injury [4, 5]. Importantly, this molecule is increased in the context of inflammation, both in human MS and in its animal model, called EAE [3]. We showed that GPR17 is upregulated in MS plaques as compared to the white matter from non-neurological donor samples and normal appearing white matter from MS donors [3]. These observations suggest that GPR17 may serve as a potential therapeutic target for myelin repair in the CNS. At present, the signaling pathway mediated via GPR17 to block OPC differentiation is not fully understood. The proposed projects aimed at understanding biology of GPR17 signaling in animal models of MS with an emphasis on developing novel therapeutic approaches for promote remyelination in the CNS.

BODY

This is a two-year study with the following two specific aims:

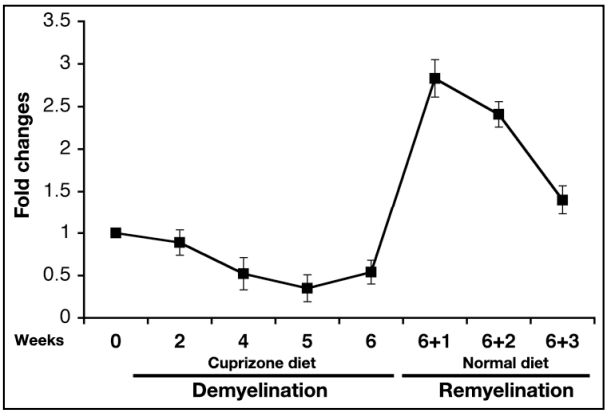
1. To delineate the role of GPR17 in murine models of demyelinating diseases.
2. To test the therapeutic potential for GPR17 agonists and antagonists in two models of MS.

As outlined in the statement of work (SOW), Dr. Lu's laboratory is responsible for experiments addressing the cuprizone-induced demyelination model of MS and analyzing the control and GPR17 knockout mice in response to EAE. Following are our research results according to the tasks outlined in the SOW.

Task 1. Test the prediction that loss of GPR17 will diminish demyelinating pathology.

A) To delineate the role of GPR17 in murine models of demyelinating diseases, we have carried out cuprizone-induced demyelinating/remyelinating study. Wildtype control mice and GPR17 null mice at age of 8 weeks were fed with cuprizone diet (0.2% cuprizone diet) for 1-6 weeks to cause demyelination and then switched to normal diet for 1, 2 and 3 weeks to promote remyelination. We first evaluated the dynamics of GPR17 expression during various stages of disease in control mice and found that GPR17 is substantially upregulated at the onset of remyelination as shown in Figure 1.

Figure 1. Correlation of GPR17 expression with progression of remyelination in the cuprizone-induced demyelinating model.
GPR17 expression detected by qRT-PCR using corpus callosum tissue from cuprizone treated wildtype animals (n=3) during 2, 4, 5 and 6 weeks of cuprizone treatment, and after animals were fed with normal diet for one (6+1), 2 (6+2) (6+2) and 3 weeks (6+3).



B) To determine the role of GPR17 in remyelination, we have examined the wildtype control and GPR17 null mice over the course of cuprizone-induced demyelination and remyelination process. Our initial study with study of a cohort of mice (10 mice in each group) show that deletion of GPR17 leads to more resistance to demyelination in the animal treated with cuprizone, consistent with previous report that blocking GPR17 activity protected MCAo-induced brain injury [4, 5] (Figure 2). In addition, appearance of Mbp expression during the remyelination process appears to be accelerated in GPR17 knockout animals as compared to control (Figure 2). These results indicate that elimination of GPR17 enhances remyelination after cuprizone-induced demyelinating injury, and support our underlying hypothesis.

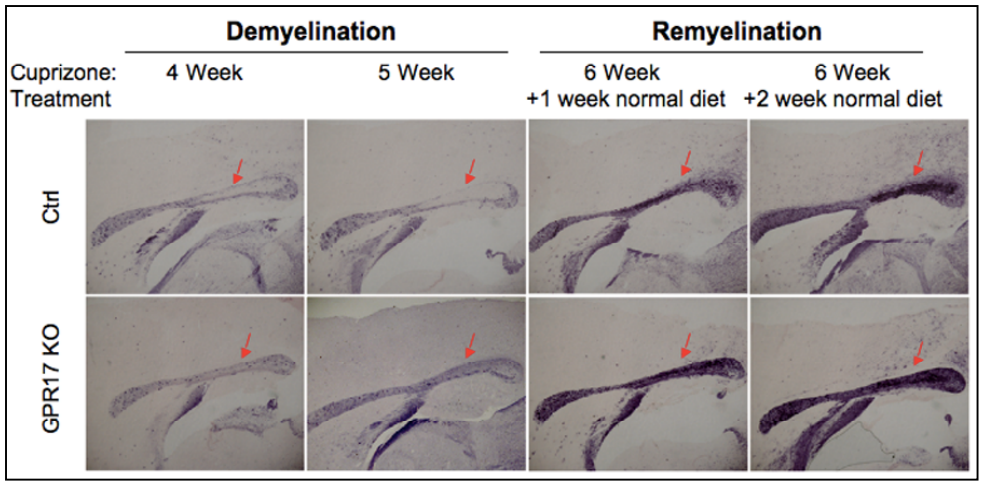


Figure 2: GPR17 knockout results in a protection against cuprizone-induced demyelinating injury and enhances remyelination
8 week old control (GPR17+/+) and GPR17 -/- knockout animals were treated with 0.2% cuprizone diet for demyelination as indicated. Animals were returned to the normal diet after 6 week treatment. The brains were harvested and processed for in situ hybridization to detect myelin marker myelin basic protein (*Mbp*) mRNA. In control mice, severe demyelination induced by cuprizone treatment as a sign of lack of *Mbp* expression in the caudal corpus callosum was observed, in contrast, *Mbp* expression in *GPR17-/-* mice is relatively maintained in the corpus callosum region, indicating a resistance to cuprizone-induced demyelinating injury in the absence of GPR17. Importantly, during the remyelination phase, more robust *Mbp* expression in the corpus callosum was detected in GPR17KO animals as compared to the control.

Task 2. To test the therapeutic potential for GPR17 agonists and antagonists in vitro and in vivo

2A) To test the therapeutic potential for GPR17 agonists and antagonists in vitro. As stated in the Task 2a (In vitro modeling of GPR17 blockade) in SOW, we obtained candidate GPR17 agonists and antagonists and began to treat oligodendrocyte precursor culture with them. Treatment of GPR17 agonists (e.g. leukotriene $LTD_{4)}$ was found to block OPC differentiation in culture (Figure 3), while treatment of GPR17 antagonists (e.g. Montelukast) appear to enhance OPC differentiation.

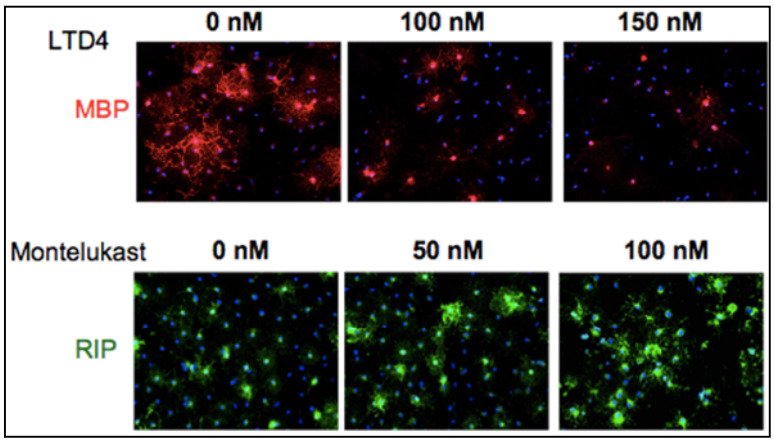


Figure 3: Effects of GPR17 agonists and antagonists on oligodendrocyte differentiation in vitro
(Upper panel) Rat OPCs isolated from neonatal pups were cultured in a differentiation condition (Sato medium supplemented with 15 nM thyroid hormone T3 and 10 ng/ml ciliary neurotrophic factor) and treated with a GPR17 agonist LTD4 at indicated concentrations for 5 days. Cells were stained with anti-MBP antibody. (Lower panel) Rat OPCs were cultured in the OPC growth medium in presence of 10 ng/ml PDGF-AA and treated with a GPR17 antagonist Montelukast at indicated concentrations for four days. Cells were then stained for differentiated oligodendrocyte marker RIP.

2B) Task 2b: **To test the therapeutic potential for GPR17 agonists and antagonists in vivo**

Testing GPR17 agonists and antagonists in vivo. We are treating the GPR17 agonists (leukotrienes LTD_4) and antagonists (Montelukast) in the context of cuprizone induced demyelination animals, and to examine the effects of GPR17 agonists and antagonists on remyelination process. We are currently optimizing the drug dosage for the study. We are still in the process to analyze the phenotypes of animals. However, these studies took longer than we expected and turned out to be overambitious. We are planning to develop a longer-term grant application based on the preliminary data generated from this application.

2C) Evaluation of CNS pathology of GPR17 null mice in response to EAE

The study from our Co-PI (Dr. Nitin Karandikar) demonstrate that GPR17-/- mice develop significant lower EAE severity. To determine the pathology in the CNS of experimental groups, we analyzed spinal cord pathology of wildtype, GPR17+/- and GPR17 -/- experimental mice after EAE induction with myelin oligodendrocyte glycoprotein (MOG) derived peptide-MOG35-55. In the WT groups, inflammatory demyelinating lesions in the dorsal and ventral white matter were associated with prominent inflammatory infiltrate (Figure 3A). Similarly, in GPR17+/- groups, we detected substantial inflammatory demyelinating lesions in the spinal white matter regions with severe loss of MBP in the lesions (Figure 4B, arrows). Strikingly, in this preliminary study, the inflammatory demyelinating lesions were hardly observed in the white matter of GPR17 null mice. Expression of the myelin gene MBP is largely maintained (Figure 4C). These preliminary observations are consistent with substantial reductions of disease severity in GPR17-/- mice compared to the control groups. Further study with more animals would need to substantiate the observations.

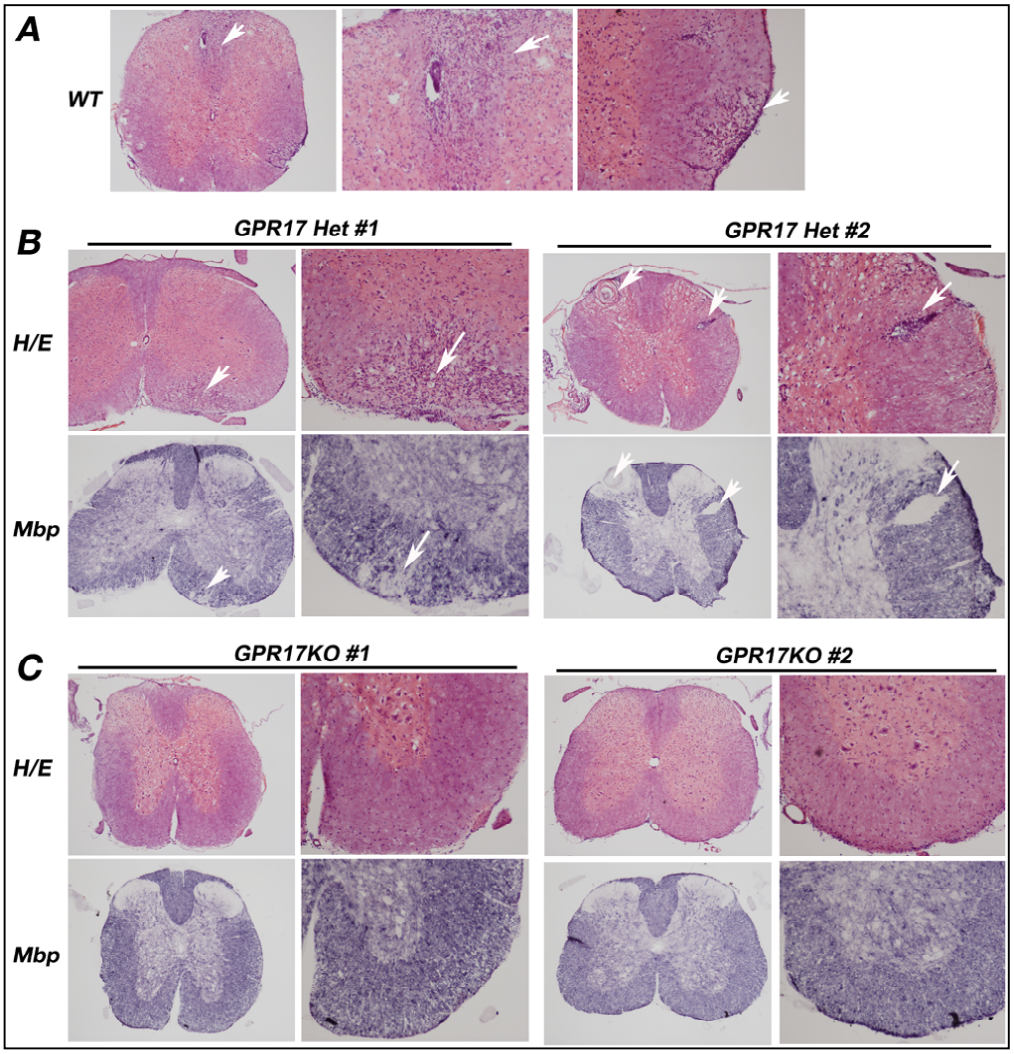


Figure 4. H/E histology analysis and MBP expression in the spinal cord of EAE mice.

Spinal cord sections from EAE mice of WT (A), GPR17+/- (B) and GPR17-/- (C) groups were subjected to H/E histology analysis and in situ hybridization for MBP. Adjacent white matter regions are shown in H/E and in situ MBP expression. Arrows indicate the control and inflammatory demyelinating lesions with enormous immune cell infiltration and corresponding MBP expression in the adjacent sections.

Key Research Accomplishments

• GPR17 expression is upregulated at the onset of remyelination during the course of demyelination/remyelination in the cuprizone-induced demyelination and EAE animal model of MS.
• Knockout of GPR17 in mice may have a protective function during cuprizone-induced demyelination injury and accelerates the remyelination process.
• The loss of GPR17 in mice may have a protective role in alleviation of the EAE-induced demyelination injury.
• GPR17 agonists appear to inhibit OPC differentiation while GPR17 antagonists enhance oligodendrocyte differentiation in culture.

Reportable Outcomes

None thus far. We anticipate multiple submissions [scientific reports and grants] in the future.

Personnel Supported by this award:
Ying Chen, PhD, Senior Postdoctoral Fellow
Tom Hu, BS, research Assistant

Conclusion

During this funding period, we have made progress to delineate the role of GPR17 in the context of demyelination/remyelination in a cuprizone-induced demyelination and EAE animal model of MS. We have determined the dynamics of GPR17 expression over the course of de/remyelination. We observed that GPR17 is upregulated at the onset of remyelination. Moreover, we showed that GPR17 knockout in mice has a protective function during cuprizone-induced demyelinating injury compared to the control mice, and enhances remyelination. Strikingly, we found that the loss of GPR17 in mice has a protective role in the EAE-induced demyelination injury, suggesting that GPR17 is an important signaling sensor that modulates demyelination injury. In addition, in vitro blocking GPR17 activity by GPR17 antagonizing drug enhances OPC differentiation and maturation. These data encourage us to pursue our study with the initial goal of developing a novel and effective strategy for myelin repair. The original plan for in vivo drug treatment study appears to be over-ambitious, we are planning to develop grant applications based on the preliminary data generated from this application to substantialize our findings.

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